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THE Journal OF THE AMERICAN LEATHER CHEMISTS ASSOCIATION

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The Journal

of the

AMERICAN LEATHER CHEMISTS ASSOCIATION

Vol. LVI MARCH 1961 No. 3

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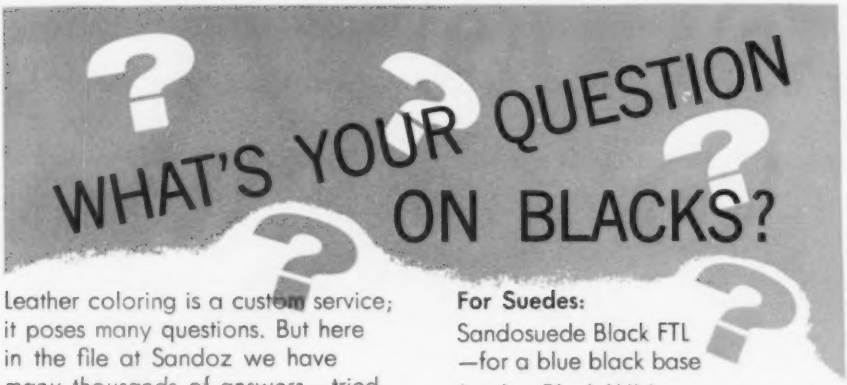

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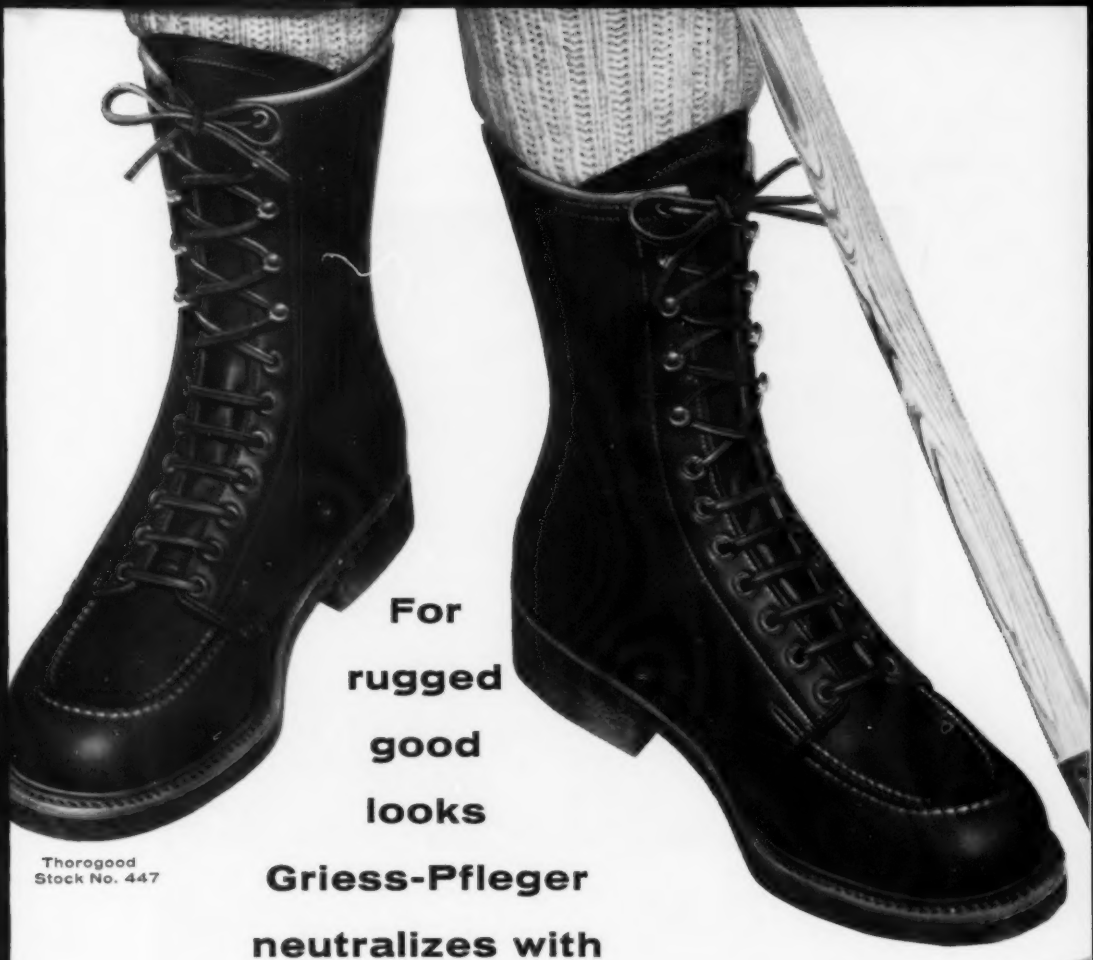

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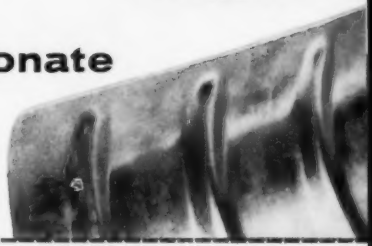

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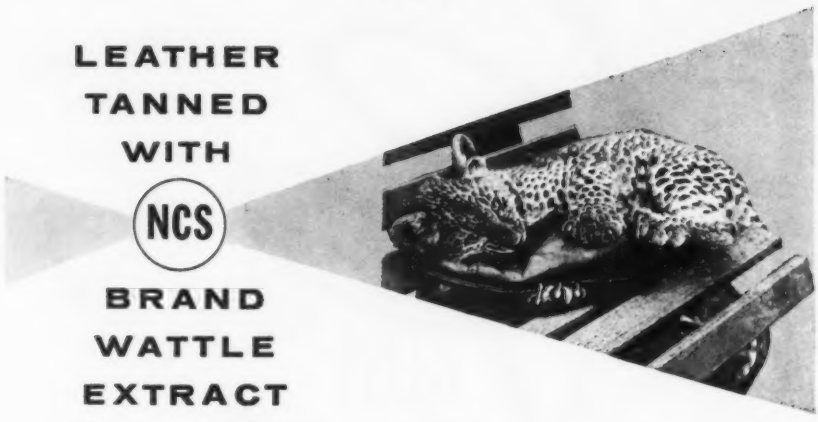

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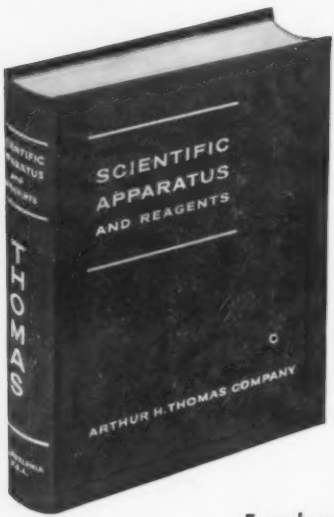

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A STUDY OF A *CLOSTRIDIUM* BACTERIUM THAT CAUSES SEVERE HIDE DAMAGE*

JEAN J. TANCOUS

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ABSTRACT

A bacterium taken from salt-cured hide samples having severe grain damage has been isolated and identified as a probable strain of *Clostridium capitovale*. This bacterium is able to tolerate up to 8% salt, and its resistant spore is able to lie dormant in dirty salt and in hide even after liming. It causes severe damage to hides in a pack and can completely digest corium collagen samples in test tube trials. The enzyme that this bacterium produces has been isolated, and its pH and temperature requirements for digesting native collagen are comparable to those of the collagenase of *Clostridium histolyticum*. The biochemical and morphological characteristics indicate that this bacterium is not *Cl. histolyticum*.

INTRODUCTION

The fact that some bacteria attack hides and skins and cause a considerable amount of leather devaluation is well known in the tanning industry. Often samples of hides and skins which show bacterial damage have been submitted to the Tanners' Council Research Laboratory for examination. Of the many organisms seen in damaged hide samples, both salt-cured and limed, one bacillus more than any other organism has occurred frequently. Because of its frequent occurrence and the severity of the damage, this organism was isolated, identified, and studied.

ISOLATION AND IDENTIFICATION

The isolation of this organism was conducted with the aid of Dr. John Wood, formerly associated with the Bacteriology Department at the University of Cincinnati. The offending organism was obtained only after many trial iso-

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lations and reinoculations of hide samples. Then single colony isolations were made three times to be reasonably sure that the cultures were not contaminated.

LIFE LINE

Mrs. Jean Jacobs Tancous holds both B.S. and M.S. degrees from the University of Cincinnati. Since 1949 she has been a Research Associate in the Tanners' Council Research Laboratory at the University, working chiefly on the microscopy and microbiology of skin and hide. She has contributed frequently to this journal.

It has been difficult to determine the identity of the organism. Many tests conducted to study its biochemical and morphological characteristics were made independently by the Tanners' Council Research Laboratory; by Miss Ruth Holtz and Mr. Edward Hill, who are associated with the Cincinnati General Hospital Surgical Bacteriology Laboratory; and by Dr. L. S. McClung, who is associated with the Department of Bacteriology, Indiana University. The combined results are as follows:

Morphological characteristics

Gram positive bacillus, 3.5 to 7 microns in length
Motile
Spore former
The oval spores swell the vegetative rod and are subterminal to terminal

Biochemical characteristics

Anaerobic, no growth on aerobic slant
Cellulose reaction—negative
Milk—slowly coagulated (about 1 month)
Gelatin—liquifies in less than 48 hr.
Nitrates to nitrites—negative
Indole—negative
Skatole—negative
Egg albumen and meat not darkened
Little, if any, H_2S formed in any reaction tested
Nonhemolytic
Egg albumen not digested
Growth on egg yolk agar—irregular, slightly roughened, dry, colorless with no precipitate or sheen obvious
Sugars fermented—dextrose, galactose, and fructose
Sugars not fermented—lactose, xylose, sucrose, inulin, arabinose, mannitol, maltose, salicin, mannose, trehalose, rhamnose, cellobiose, glycerol, and starch
Nonpathogenic—intraperitoneal inoculation of mice with heart infusion culture supernatant

The bacillus automatically falls in the *Clostridium* group since it will not grow on an aerobic slant. Thus the characteristics of this organism were compared with those of the *Clostridia* listed in *Bergey's Manual of Determinative Bacteriology* (1). The identity of this organism does not seem to fit, in all respects, the identity of any *Clostridium* listed. The organism seems related to *Clostridium captivale*, and thus it has been decided that it should be listed tentatively as a strain of this species.

HIDE DAMAGE STUDIES

The damage this bacillus causes to hides and skins in storage is of concern to the tanning industry; therefore the ability it has to attack hides as they occur in a pack was investigated. Its level of salt tolerance was checked by the following method: Hide samples were brined in a 10-to-1 float ratio of 4, 6, 8, 10, 12, and 14% salt solutions. An excess of brine was used so that the water of the hide could come to equilibrium with the brine without causing more than a small dilution of the concentration of salt in the brine. After the hide samples were treated for 48 hr., they were drained and cut in two. The divided samples were inoculated on the flesh side and placed flesh to flesh in a petri dish. A control containing no added salt was also tested. After the seven samples, in individual petri dishes, were held at room temperature for one week, they were examined microscopically (2) for bacterial growth. It was found that the organism could not grow in hide samples that were treated with brines containing 8% NaCl or more. Samples that were treated with brines containing 6% salt or less were attacked by the bacillus.

The degree to which the bacteria could digest the hide was then checked. Samples were brined at the 4% and 6% salt levels as previously described, a variation in the procedure being that the one specimen was cut into approximately 1-gram pieces before it was placed flesh to flesh with the inoculated bottom hide sample which was left uncut. The samples in their separate petri dishes were stored in plastic bags to prevent moisture loss. Two pieces were taken from each dish after 3, 7, 10, and 14 days and after 7½ weeks. One was analyzed for the amount of hide substance digested, and the other was checked microscopically for bacterial growth. Microscopic examinations were used to observe the bacteria involved in the digestion, because the hide samples could not be autoclaved. The bacteria in question were apparent in large numbers throughout the cross sections of both the test samples.

The samples taken for digested protein were enclosed in small bottles with 10 ml. of distilled water. The bottles were shaken vigorously in a machine for one half hour. The water-soluble protein was then separated from the insoluble protein by filtering the sample through glass wool. The filtrate and the residue were each analyzed for nitrogen by the Kjeldahl method. The two nitrogen fractions were added together; the filtrate nitrogen was divided by the total nitrogen for calculating the digested protein nitrogen.

The graph showing the percentage of hide substance digested by the bacteria plotted against time is given in Fig. 1. It can be noted that in a 3-day period there was a considerable amount of damage, particularly at the 4% level of salt, where 26% of the hide substance was digested. At the 6% level, 18% of the hide substance was digested in the 3 days. The curves in both cases leveled off, and by the end of 10 days the bacteria had digested almost a third of the hide substance. No more digestion occurred after this point even after 7½ weeks, presumably because the by-products of its growth caused the organism to sporulate.

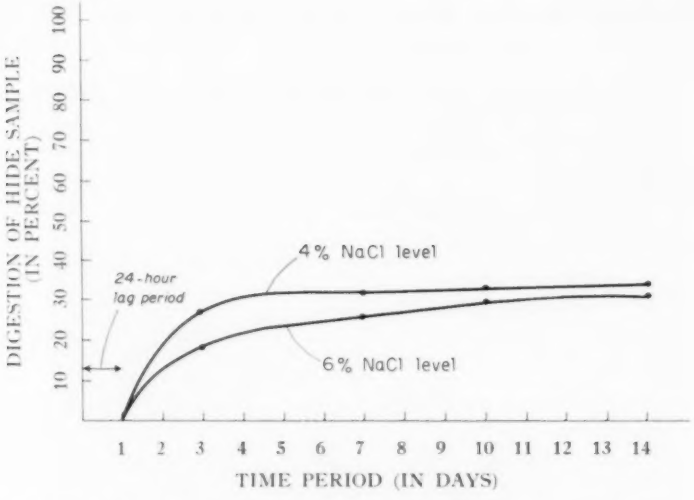
DIGESTION OF HIDE SAMPLE (IN PERCENT)
10 20 30 40 50 60 70 80 90 100
4% NaCl level
24-hour lag period
6% NaCl level
1 2 3 4 5 6 7 8 9 10 11 12 13 14
TIME PERIOD (IN DAYS)

FIGURE 1.—Graph showing the digestion of hide samples treated with 4% and 6% NaCl and inoculated with *Clostridium capitovale*.

A study was then made to determine whether this organism could completely digest corium hide collagen when the by-products of its growth were removed. The corium hide collagen was prepared as follows: A 75-lb. heifer hide was taken immediately to the laboratory from the slaughterhouse, cut into 1-ft.-square pieces, and stored in sealed plastic bags in a deep-freeze unit until it was needed. For testing, a thick bend portion of the hide was taken, partially thawed, and split horizontally into 3 areas, namely the epidermal, corium, and flesh areas. Only corium collagen was saved, and it was considered to be as native as feasibly possible.

The digestion study was conducted as follows: Approximately 2-g. samples of native corium collagen and 20 ml. of 4% sodium chloride solution were put

into test tubes which were made anaerobic by treating the cotton plugs with pyrogallol and Na_2CO_3 and sealing the tubes with rubber stoppers. Four samples were used as controls; four were inoculated. The liquid was removed every 2 or 3 days and was analyzed for nitrogen colorimetrically with Nessler's reagent (3). A fresh 4% solution of sodium chloride was replaced in the test tubes which were again made anaerobic. After the inoculated collagen was completely digested, the individual fractions of nitrogen were added together, and each fraction was divided by the total nitrogen to get percentage digestion.

The graph showing the percentage of hide substance digested by the bacteria plotted against time is given in Fig. 2. The graph shows that when

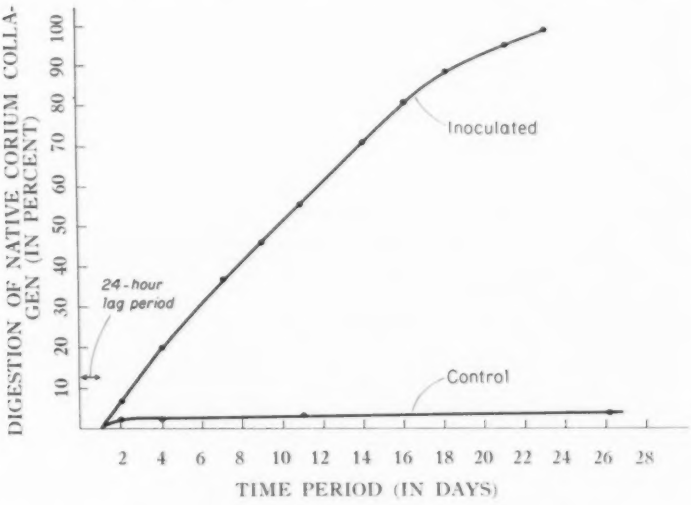
DIGESTION OF NATIVE CORIUM COLLAGEN (IN PERCENT)
10 20 30 40 50 60 70 80 90 100
Inoculated
24-hour lag period
Control
2 4 6 8 10 12 14 16 18 20 22 24 26 28
TIME PERIOD (IN DAYS)

FIGURE 2.—Graph showing the digestion of native corium collagen inoculated with *Clostridium capitovale*. Conditions: 4% NaCl solution; room temperature; anaerobic tubes.

the digested protein is removed and new saline is replaced, the digestion is a straight line function of the time and is complete after 23 days. As can be seen by the bottom plot on the graph, the uninoculated controls showed less than 5 per cent digestion in the same time period. Although not shown on the graph, it was decided that the control tubes should be inoculated after the many changes of saline. When this was done, there was no digestion of the collagen. Since the organism needs the nutrients that were extracted to get a start, a few drops of the white and yolk of a chicken egg were added to each tube. After these were added and the tubes were reinoculated, the

collagen was digested in 2½ weeks. The fact that these materials initiated the reaction indicates that certain nutrients are essential for the growth of bacteria in hides and skins.

In the testing of the native corium collagen, as in the testing of hide samples, autoclaving could not be used. A periodic check with a microscopic examination was the means used for knowing whether or not the controls and digestions were contaminated. Except for a small amount of hide surface contamination that occurred at the time of preparation, the samples were free of unwanted organisms. The 4% salt and the anaerobic conditions aided in preventing contamination.

A highly magnified collagen fiber bundle as it appears when being attacked by the bacillus is shown in the photomicrograph in Fig. 3. It is interesting

FIGURE 3.—A photomicrograph of a collagen fiber bundle in a cross section of steerhide being attacked by *Clostridium capitovale*. × 970.

that the organism grows along the inside of the collagen fiber bundles and not across the fibers. Some long vegetative rods can be seen just below the center of the illustration; however, most of the organisms are present as spores.

ENZYME STUDIES

An organism that can attack native collagen as rapidly as this one must certainly produce a collagenase. According to Boyd, Logan, and Tytell (4) and Mandl, MacLennan, and Howes (5) the enzyme of *Clostridium histolyticum* can be salted from the growth medium with ammonium sulfate. A modified method was used for preparing the *Cl. capitovale* enzyme.

The bacteria were grown anaerobically in 10-ml. to 15-ml. portions of thioglycollate gelatin which was made with 4 g. NaCl, 7.6 g. BBL Thiogel Medium, and 100 ml. water. The bacteria were allowed to grow for one week at room temperature. They were filtered from the medium by means of a Berkefeld filter. The filtrate was saturated with ammonium sulfate using approximately 60 g. $(NH_4)_2SO_4$ per 100 ml. filtrate and was placed in a refrigerator for 3 to 7 days to allow the enzyme to precipitate. The enzyme precipitated well without any interference. A Buchner funnel, with No. 42 Whatman filter paper, was used to collect the enzyme which was then redissolved in water, dialyzed, and dried in petri dishes over $CaCl_2$ in desiccators. The dialyzing and drying procedures were done in a refrigerator so that the enzyme would not be unnecessarily inactivated by warm temperatures. The dry enzyme was scraped from the dishes and stored in a refrigerator until it could be tested. The yield of enzyme thus prepared was slightly over a tenth of a gram per liter of medium.

The *Cl. capitovale* enzyme was tested for its optimum substrate-to-enzyme ratio, its optimum pH, and its optimum temperature. A commercial preparation of the *Cl. histolyticum* enzyme was obtainable from Agricultural Biologicals Corporation, thus in all tests the *Cl. histolyticum* enzyme was also used so comparisons between the two enzymes could be made. The method of testing the enzyme activity was essentially that used by Springell (6).

OPTIMUM SUBSTRATE-TO-ENZYME RATIO

Portions, 2, 4, 8, 12, and 16 mg., of the *Cl. histolyticum* and *Cl. capitovale* enzymes were checked for their digestive ability. Samples of native corium collagen approximately 50 mg. in weight were sliced on a freezing microtome set at 50 microns. The collagen was placed in small centrifuge tubes with the enzyme and with 3 ml. of pH 7.6 buffer which was made by mixing M/5 boric acid and M/20 borax. A control sample which contained no enzyme was also checked for digestion. After a 24-hr. digestion period at a temperature of 37° C. the tubes were cooled in ice water and 1½ ml. of cold 15% trichloroacetic acid was added to stop the action of the enzyme and to precipitate undigested protein. The tubes were centrifuged, the supernatant was decanted into a Kjeldahl flask, and the residue was washed with about 5 ml. of cold 5% trichloroacetic acid. The tubes were again centrifuged, and the wash supernatant was added to the first supernatant. The residue was transferred to another Kjeldahl flask. The two fractions were digested for 2 hours with 5 ml. concentrated H_2SO_4 containing 1% selenium dioxide. Nitrogen was determined colorimetrically with Nessler's reagent. Percent digested protein nitrogen was calculated: the supernatant nitrogen minus the enzyme nitrogen, multiplied by 100, was divided by the supernatant nitrogen plus the residue nitrogen minus the enzyme nitrogen.

The digestion curves of native corium collagen brought about by various amounts of the *Cl. capitovale* enzyme and the *Cl. histolyticum* enzyme are

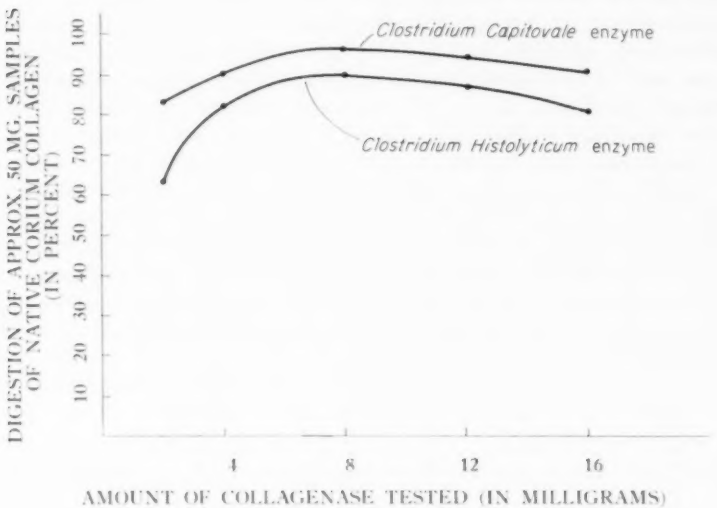

FIGURE 4.—Graph showing the digestion of native corium collagen obtained with the collagenases of *Clostridium capitovale* and *Cl. histolyticum*. Conditions: pH, 7.6; temperature, 37° C.; time, 24 hr.

plotted in Fig. 4. The graph shows that in the 24-hour digestion period the curves for the two enzymes are very nearly comparable, both curves having their optimum digestion around 8 mg. per 50 mg. of collagen. It would appear from the graph that both enzymes digest approximately 90% of the collagen at the optimum range, but the graph should not be used for comparing the two enzymes as to their activities. From studying the graph it was decided that the 10 mg. of enzyme recommended by Springell could be used as a satisfactory amount for testing 50 mg. collagen. The use of the borate buffer and cold trichloroacetic acid was also satisfactory, as there was only 3.5% digestion in the control, indicating that these reagents were not causing unwanted digestion of the collagen.

OPTIMUM pH

To check the optimum pH, the samples of native corium collagen were prepared as described above and placed in the centrifuge tubes with 10-mg. portions of enzyme and 3 ml. of borate buffer. Buffers were prepared from M/5 boric acid and M/20 borax with pH values ranging from 5.6 to 7.8.

Control samples which contained no enzyme were also checked. After a 6½-hour digestion period at a temperature of 37°C. the reactions were stopped, and the amount of digestion was determined as mentioned above. The results of this test indicated that the *Cl. capitovale* enzyme was considerably more active than the commercially prepared *Cl. histolyticum* enzyme. Since the method of preparing the *Cl. capitovale* enzyme consisted of drying the material over $CaCl_2$, and since it was known from previous analyses that 2 to 6 mg. of calcium sulfate (0.6 to 1.8 mg. calcium) accelerates the reaction, the determinations were repeated with 6 mg. of $CaSO_4$ being added to each tube. A 4-hour digestion time was used. The results of this test indicated that the *Cl. capitovale* enzyme was, as before, more active than the commercial enzyme but, as anticipated, the difference was not as great. These tests, conducted at the various pH values, indicated that the two enzymes had similar pH curves and had increasing digestions with increasing acidity as shown in Fig. 5. The controls showed less than 5% digestion at all pH values tested.

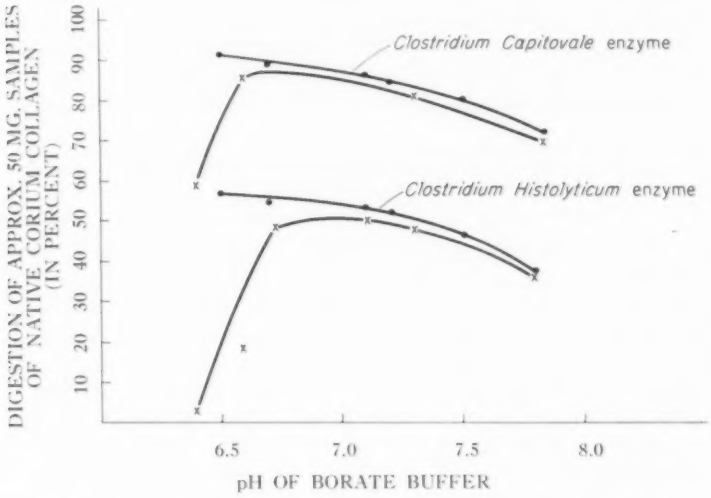

FIGURE 5.— Graph showing the digestion of native corium collagen obtained at various pH values with the activated collagenases of *Clostridium capitovale* and *Cl. histolyticum*. Conditions: time, 4 hr.; temperature, 37° C.; $CaSO_4$, 0.006 g.; enzyme, 0.010 g.; enzyme pretreatment: AB = none (•), A′ B′ = 2 hr. (×).

To determine the optimum pH value it was decided that the enzymes should be pretreated with activator at the various pH levels for a 2-hour period prior to the addition of the collagen. This pretreatment affected the activities

of the enzymes considerably in the pH levels below 6.6 but did not seem to affect them between the values of pH 6.7 and 7.8. The results, plotted in Fig. 5, indicate that the optimum range for both enzymes lies between pH 6.7 and 7.4. Below 6.7, and particularly at pH 6.4, the enzymes are rapidly inactivated by acidity. From the data a pH of 7.1 is considered to be the optimum pH for the *Cl. capitovale* enzyme activity.

OPTIMUM TEMPERATURE

The optimum temperature for digestion was then checked. The collagen was prepared and placed in centrifuge tubes with 10-mg. portions of enzyme, 6 mg. of $CaSO_4$, and 3 ml. of pH 7.1 borate buffer. Six temperatures ranging between 30° and 47°C. were used, and it was found that in a 1½-hour digestion period there was an increase in activity with increase in temperature. At 30°, 34°, 37°, 40°, 44°, and 47°C. the digestion was 5.3, 24.5, 47.4, 53.7, 61.8, and 93.8% respectively for the *Cl. capitovale* enzyme and 3.5, 6.4, 4.2, 13.1, 15.5, and 42.7% for the *Cl. histolyticum* enzyme.

A second test was then made using a ½-hour digestion period and temperatures between 37° and 55°C. The results are plotted in Fig. 6, and again there is an increase in activity with increase in temperature for both enzymes,

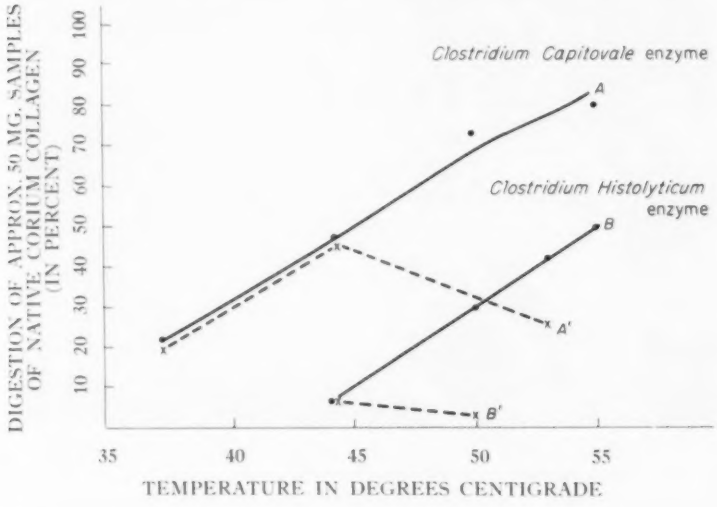

FIGURE 6.—Graph showing the digestion of native corium collagen obtained at various temperatures with the activated collagenases of *Clostridium capitovale* and *Cl. histolyticum*. Conditions: pH, 7.1; time, ½ hr.; $CaSO_4$, 0.006 g.; enzyme, 0.010 g.; enzyme pretreatment: AB = none (—), A′ B′ = 2 hr. (--).

as shown by plots A and B. Control digestions were also checked, and except at 55°C. there was less than 5% digestion.

To determine the optimum temperature it was decided that the enzymes should be treated at the various temperatures for a two-hour period prior to the addition of the collagen. When the enzymes were pretreated, there was a marked decrease in the activities of both enzymes at temperatures of 50°C. and above. At 44°C. there was no difference as shown by plots A′ and *B′*, which were designated by broken lines, since the data are not sufficient for showing the exact curves. From plots *A′* and *B′* it can be seen that for long digestion periods a temperature of 37°C. can be considered as an optimum temperature, whereas temperatures somewhat higher could be safely used when short digestion periods are used. These data are in agreement with the known effect of temperature on many enzymes (7).

HIDE DAMAGE AND ITS PREVENTION

It has been shown that the *Cl. capitovale* strain is a collagenase producer and is therefore capable of destroying hide substance. Figure 7 shows a piece of

FIGURE 7.—Salt-cured hide with severe grain damage from which the *Clostridium capitovale* organism was isolated.

salt-cured hide from which the organism was isolated. The sample shows hair slip and grain damage that involves the epidermal area so severely that the underlying corium is exposed. Areas such as these contain the bacteria in great quantities throughout the entire thickness of the stock. Figure 8

FIGURE 8.—The grain of sole leather showing that the bacterial damage is quite deep and that the underlying corium fibers are exposed.

shows the grain of sole leather that was made from stock that was also damaged. Although the pressure of rolling the leather seems to have reduced the effect, the damage is quite deep and the underlying corium is exposed.

From our work with the *Cl. capitovale* organism important information has been collected. This organism can, and does very seriously, damage hides in cure and in storage, and the hide devaluation is certainly undesirable. When this organism grows in hides, it is evident that the salt level must have been below 8%, which is much below a good cure level and indicates a poor application of salt, washing out of the salt, or inhibition of the salt penetration because the flesh had not been removed. Finding the organism in salt-cured hide means that the areas were definitely undercured. Finding the organism in limed hide—which has actually been observed—means there has been undercure or improper soaking of contaminated hides in the tannery.

This organism is able to lie dormant in dirty salt and in limed hides because the spore form is resistant to salt, lime, and low moisture levels. It can then easily contaminate and bring about damage to hides and skins when dirty salt is used repeatedly and when contaminated hides remain too long in the soak water.

Although this organism appears frequently, there is no doubt that its control is not difficult. When hides and skins are properly cured with sufficient salt, the organism is inhibited. This bacillus can be kept in check, too, when hides are properly handled so the salt is not washed out during cure and storage, and when soak waters are kept cool, short, and disinfected. If a hide or skin is damaged by the bacillus, it is a definite sign that the salt has dropped below 8% and that the lag period of 24 hours has been surpassed.

SUMMARY

1. A bacillus that causes severe hide damage has been isolated and has been identified tentatively as a strain of *Clostridium capitovale*.

2. The *Clostridium capitovale* organism will tolerate up to 8% sodium chloride and is resistant in spore form.

3. This bacillus produces a collagenase which has an optimum pH of 7.1 and an optimum temperature of 37°C.

4. Damage by this organism can be prevented by proper curing and proper handling of hides and skins.

ACKNOWLEDGMENT

Grateful acknowledgment is given to Mr. Fred E. Artz, Howes Leather Company, Inc., who submitted the damaged hide and leather samples used in this research. The author also expresses thanks to Dr. John Wood for his guidance in isolating and identifying the organism; to Mr. Bob Sojgren for advice in preparing media; to Mr. Ed Hill, Miss Ruth Hotz, and Dr. L. S. McClung for their individual studies of the organism and help in assigning a name; and to members of the department, Mr. W. T. Roddy, Dr. Fred O'Flaherty, Dr. Clara Deasy, and Dr. Robert Delcamp for their guidance and criticism. The technical assistance of Miss Audrey Douglas, Miss Sue Marcus, and Miss Carmen Bereskin has been important in completing the enzyme studies and is sincerely appreciated.

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DISCUSSION

DR. T. C. CORDON: The matter of damage to hides at the time of curing has occupied people in the industry ever since there was such an industry. Just what causes this damage is something else again. We know there are

bacteria and other microorganisms which attack collagen; otherwise the collagen would accumulate. But as Mrs. Tancous pointed out, there are only a very few bacteria which have been shown to produce a true collagenase. She mentioned two of them and has now found another one.

Many people have worked on this problem. In our laboratory a few years ago we attacked this problem from a little different standpoint. We looked for bacteria which produced enzymes that attacked collagen, but we looked only in places where the salt content was high. The halophilic bacteria we isolated were not able to produce a strong collagenase. We felt there was a synergistic effect—that two or three organisms, or perhaps three or four, might be able to decompose collagen, whereas single pure cultures could not.

I think we owe Mrs. Tancous a vote of gratitude for pointing out again something we all should know, that it is the improperly cured hide that spoils. This is a perpetual problem that is always with us. It has been very much in the forefront in recent years because of the large number of hides which we ship to foreign lands. They are often subjected to rigorous conditions during shipment.

From what I have read, they sometimes get into foreign countries in very bad condition. Here is an indication that when damage occurs it is because the hides were not treated properly. If they are cured properly, this organism certainly will not attack them.

Now as to specific questions on this paper, I would like to ask Mrs. Tancous about the occurrence of the organism. How often have you found this organism? Is it really widespread? Does it occur in brine-cured stock? Does it occur in other places in hide processing? Has it caused damage in places other than in the cure?

MRS. TANCOUS: I believe that any hide that contains less than 8% salt can develop this organism. This can easily happen in the hide cellars or even in the cure or in the soak—any place before the liming operation. It can occur if the organism is there, and if the level of salt drops below 8%. After liming, the organism goes into spore form and probably will not cause trouble after that stage. But before liming and in any condition where there is less than 8% salt and where there is a time period of two or three days, this organism, if it is present, can certainly cause damage.

DR. CORDON: A question now about the production of the enzymes. You did not state the nature of the medium on which you grow this bacteria for producing the enzyme. Could you clarify that for us?

MRS. TANCOUS: It is a thioglycolate gelatin medium, and we have learned since we have done this work that other collagenase-producing organisms are grown on proteins that are simpler than gelatin. We are quite amazed

that ours is that much different and that it can use gelatin to give a collagenase; and apparently it is an active collagenase.

DR. CORDON: There are a great many bacteria that can attack gelatin. But I was wondering, as a matter of academic interest, whether something in the nature of collagen or gelatin is necessary in the medium for the organism to produce the enzyme. In other words, whether it is constitutive or whether it is adaptive and is produced only in the presence of specific substrate.

MRS. TANCOUS: We have not tested it on the other media that other investigators have used, but we do know that it does not grow on milk and does not grow on cellulose. There are many media that give negative results with this organism. It prefers gelatin and it prefers collagen to many, many other substrates. We have not tried it on blood, albumens, or globulins, or that type of media.

C. ALLAN LAUDER (Beardmore & Co. Ltd.): You mentioned 8% salt as the minimum cure. Is that 8% on the dry weight or on the "as received" weight?

MRS. TANCOUS: On the moisture of the hide weight—but on a dry weight it would also be around 8%.

DR. H. G. TURLEY (Rohm & Haas Co.): I don't know whether you mentioned whether this organism is pathogenic or not.

MRS. TANCOUS: It is not pathogenic. We have had the toxicity tested at the University of Indiana.

DR. TURLEY: Some organisms of that type are.

MRS. TANCOUS: Many of the *Clostridia* are gas gangrene producers.

DR. TURLEY: There are a lot of interesting factors about this organism. To what extent has it been found in skins and hides? Do you think it could do a lot of damage in soaking, for example? The fact that it spores might mean that it would survive the lime treatment and probably does, and I am wondering if it is the kind of organism that has given in the past a lot of trouble with puering, where skins had water pricks. In the puering of goatskins it used to be that certain organisms were susceptible to sulfide. If this organism grows well in the SH compound, then that kind of organism would grow with amazing speed if it started working on the grain. In a few hours there would be extensive damage, and it might create a lot of other difficulties beyond the puer.

PROFESSOR RODDY: The point which you brought up a few moments ago, which Mrs. Tancous did not answer, was to what extent this organism has been found in skins and hides; and I would like to emphasize that we have run across this organism through a period of years at the University of Cincinnati. It has always been outstanding in that it is a very large type of bacillus. It has never been classified before because in the early work which was performed at the University of Cincinnati the primary interest was to eliminate such organisms. So there has been no attempt to specifically classify them until recently when this organism came to the fore and indicated it might cause amazing activity.

Now as to the point Dr. Turley mentioned with regard to the effect on goatskins and water-prick damage—in the case of water-prick damage on the skins and hides that we have examined, we usually do not run across this particular bacillus. If I remember correctly (and Mrs. Tancous will correct me if I am wrong), they are usually of the coccus variety.

MRS. TANCOUS: They have been smaller bacilli, too.

DR. JULIUS PFANNMULLER (Wallerstein Company): In the former process of puering goatskins with dog manure infusion the proteolytic action on the skins was obtained by bacteria developed under anaerobic conditions. The skins during puering were resting submerged on the bottom of the paddle, with air excluded, and after a certain time, perhaps 4 to 8 hours, a heavy fermentation occurred, caused by anaerobic bacteria, which generated proteolytic enzymes attacking and dissolving collagen.

I have observed cases where all the skins were dissolved if the fermentation was too strong and too long. I think this was due to the action of a very strong collagenase produced by these anaerobes.

According to my observations, one may obtain these anaerobic bacteria easily. Before neutralizing, simply let the skins which were subjected to a normal liming process stand for a few days submerged under water at about 37°C. In a few days one can obtain a culture of these anaerobes. In about 4 to 6 days the skins are entirely dissolved.

Further details about these anaerobic bacilli and their enzymes are described in U. S. Patent No. 2,373,410, dated April 10, 1945.

MRS. TANCOUS: When I said the bacillus would not attack after the liming process, I was assuming that the bate and the other processes would be shorter than four days. If you do have long periods of time, I am sure that the organism can grow again. Because it is a spore former, it could become vegetative. In four days' time it could do much damage.

DAVID BALFOUR (Hamblet & Hayes Co.): Considering that this is a spore-forming bacteria and very resistant, have you done work in the laboratory in Cincinnati parallel to work on anthrax control, for instance, to destroy the spore?

MRS. TANCOUS: No, we have not done that.

MR. BALFOUR: One other thing: I hope that modesty will not prevent you from giving it a name and calling it *Clostridium cincinnatium* or something of the sort.

MRS. TANCOUS: We have to use a Latin name, and we are thinking of calling it *Clostridium integumentum* which is the Latin name for skin and hide.

Scientists and engineers engaged in chemical industry research have more than doubled in the past nine years.

Now estimated by the National Science Foundation at approximately 34,000, as of January 1959, only 15,632 were employed in 1950, according to a survey by the National Research Council. At the same time the number of research technicians has grown at nearly the same rate—16,941 in 1950 compared to 34,400 today, according to these same sources.

The chemical industry has long led all other manufacturing industry in the proportion of employees engaged in research. For every 10,000 production workers, the industry employs 661 research workers as against 215 for all manufacturing industry.

—*Chemical News*, Nov.-Dec., 1960.

The chemical industry, as of January 1959, employed 36,600 engineers, 31,100 chemists, 4,200 medical scientists, 3,900 biological scientists, 1,600 agricultural scientists, 1,200 physicists, 700 metallurgists, 600 mathematicians, 300 geologists and geophysicists, and 3,000 other scientists.

—*Chemical News*, Nov.-Dec., 1960.

GELATIN DEGRADATION BY MICROBIAL AND OTHER ENZYMES AS RELATED TO THEIR UNHAIRING ACTIVITY*

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ABSTRACT

A study of the enzymatic depilation of cattlehide would be aided materially if an assay method could be found which correlated with hair-loosening action. Enzymes from bacteria, fungi, plants, and animals were evaluated according to their action on gelatin as measured by formol titration, decrease in viscosity, and liquefaction. Although the correlation between these assay methods and the degree of hair loosening was statistically significant, the magnitude of the relationship was not great enough to permit dependable predictions.

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INTRODUCTION

In a study of the enzymatic depilation of hides and skins attempts have been made to find an assay procedure which would correlate with the depilatory action of the enzymes. Such an assay would greatly facilitate the work on unhairing and would lead to a better understanding of the mechanism of the depilatory action. Attempts to correlate hair-loosening activity of enzymes with action on casein and starch (1) and elastin (2) have been reported previously. Although neither gelatin nor its precursor, collagen, has been implicated in the depilatory action of enzymes, some workers believe that there may be a correlation between the ability of some enzymes to attack gelatin and their ability to cause the loosening of hair on animal hides. In order to check this we have assayed enzymes of plant, animal, and microbial origin by three different procedures using gelatin as the substrate. The results are reported here.

*Presented in part at the 60th Annual Meeting of the Society of American Bacteriologists, Philadelphia, Pennsylvania, May 1–5, 1960.
†Life Lines of all the authors were published in last month's *Journal*.
‡Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

MATERIALS

Enzymes.—The enzymes studied were supplied by the firms listed in Table I. The abbreviations given there are also used in the tables that follow. These enzymes were used as received without further purification.

TABLE I

LIST OF THE SUPPLIERS OF THE ENZYMES USED IN THESE STUDIES

Code	Source of Enzyme
MC	Miles Chemical Co., Clifton, N. J.
RH	Rohm & Haas Co., Philadelphia, Pa.
PB	Pabst Brewing Co., Milwaukee, Wis.
BS	Biddle-Sawyer Co., New York, N. Y.
NB	Nutritional Biochemicals Corp., Cleveland, Ohio
VC	Viobin Corporation, Monticello, Ill.
WC	Wallerstein Co., Staten Island, N. Y.
IM	Institute of Microbiology, New Brunswick, N. J.

Gelatin.—In order to obtain reproducible results it was necessary to use gelatin from one source. Even different lots from the same manufacturer had to be carefully checked. Gelatin supplied by the Amend Drug and Chemical Company* was used in all three assays.

Buffers.—The following buffers were used for maintaining the pH at the desired values:

pH 5.0 McIlvaine's citric acid–phosphate
pH 7.0 Mixture of $Na_2H\ PO_4$ and NaH_2PO_4
pH 8.5 Tris (hydroxymethyl) amino methane

The pH was held within about 0.1 unit throughout the reactions.

Formol titration.—In the formol titration assay 0.5-g. portions of gelatin were weighed into 50-ml. Erlenmeyer flasks, and 8 ml. of water and 2 ml. of buffer were added. The flasks were held in a water bath at 40°C. until the gelatin dissolved. Five ml. of enzyme solution was then added, and incubation was continued at 40°C. for one hour. Ten ml. of 37% neutralized formaldehyde was then added. In the case of the blanks the formaldehyde was added before the enzyme. The solutions were titrated to a faint pink color, using 0.1*N* NaOH and phenolphthalein as the indicator. Results are reported as milliequivalents of nitrogen per g. of enzyme. At pH values other than 7.0 the procedure was modified to the extent that gelatin solutions were adjusted to the desired pH with the buffer and aliquots pipetted into the flasks.

*The mention of trade names or companies does not constitute an endorsement by the Department of Agriculture over other products or companies not mentioned.

Liquefaction.—In this procedure gelatin solutions were made in the buffer solutions so that the concentration in the reaction mixture (33 mg./ml.) was the same as that used in the formol titration assay. Enzyme solutions were diluted in 0.1% intervals, and one ml. from each dilution was placed in each of two test tubes. Four ml. of gelatin solution was added to each tube, and the tubes were held in a water bath at 40°C. for one hour. They were then placed in ice water, and after about ½ hour the concentration of enzyme in the last tube remaining liquid was recorded. In order to facilitate comparison with the other assay results the reciprocal of this concentration in micrograms per liter was recorded in the tables.

Viscometric assay.—A 4% gelatin solution was made up in water saturated with toluene. Ten ml. of this was placed in a stoppered flask in a 40° ± 0.5°C. water bath, and 4 ml. of buffer was added. When the solution reached 40°C., one ml. of enzyme was added, and the time was noted. Ten ml. of this mixture was immediately pipetted into the wide arm of an Ostwald-Fenske-Cannon-type viscosimeter, and measurements were begun after 2½ to 3 minutes of digestion time. Four successive determinations of efflux time were made in not more than a total of 15 minutes from the moment that the enzyme solution was added. Blanks were run in the same way except that water was added instead of the enzyme. The results were plotted, and relative fluidity values were obtained from the curve at 5- and 10-minute digestion times (Fr_5 and Fr_{10} respectively). The units of activity were obtained by using the formula

$$Units/g. = \frac{Fr_{10} - Fr_5 \times 33.3}{e}$$

where e = mg. enzyme contained in 1 ml. enzyme solution as added to the gelatin solution. This procedure is empirical, and all variables must be carefully controlled.

RESULTS

In order to simplify the interpretation of the data the ratios of the values for the three assays as well as the actual values obtained are given in Tables II, III, IV, and V. If each procedure measured the same activity of the enzymes, the ratios of the values obtained by the different assays should be constant. Conversely, divergence from constant values would indicate that the procedures were measuring different activities of the enzymes. The division of the enzymes into bacterial, fungal, and plant and animal groups was done merely to simplify presentation.

Table II shows the data obtained with five of the bacterial enzyme preparations. In considering the marked differences in the activities, it should be

TABLE II

COMPARISON OF THE ACTION OF SOME BACTERIAL ENZYMES ON GELATIN AT pH 7.0

Enzyme	Code	Assays			Ratios		
		Formol Titration meq-N/g	Viscosity Units/g	Liquefaction $\frac{1}{\mu g/l}$	V/FT	V/L	FT/L
Protease 15 Concentrate	RH	44.2	81.0	20.0	1.8	4.1	2.2
Protease L-56-D	PB	38.8	68.5	25.0	1.8	2.7	1.6
Enzyme 4511-3	WC	23.0	19.0	11.0	0.8	1.7	2.1
Rhozyme H-39	RH	16.5	6.2	8.6	0.4	0.7	1.9
Bioprase	BS	8.4	0.8	4.2	0.9	0.2	2.0

borne in mind that these relative activities are dependent upon the degree of purification of the enzyme preparation, and this varied with the intended end use for a particular product. Examination of the columns giving ratios of the assay values shows marked differences between the products. The values for the ratio of viscosity to formol titration vary as much as 4.5 times, and those for viscosity to liquefaction vary over 20 times. The ratios of formol titration to liquefaction are much more consistent. The greatest difference is 1.4 times between Protease 15 Concentrate and L-56-D.

TABLE III

COMPARISON OF THE ACTION OF BACTERIAL ENZYMES FROM THE SAME SOURCE (CODE MC) ON GELATIN AT pH 7.0

Enzyme	Assays			Ratios		
	Formol Titration meq-N/g	Viscosity Units/g	Liquefaction $\frac{1}{\mu g/l}$	V/FT	V/L	FT/L
HT Proteolytic, 202	96.9	197.5	62.5	2.0	3.2	1.6
HT Concentrate "P"	94.9	165.5	33.3	1.7	5.0	2.9
HT Concentrate, 4903	71.3	123.0	50.0	1.7	2.5	1.4
HT Proteolytic, 1	55.2	136.5	33.3	2.5	4.1	1.7
HT Proteolytic, 210	53.4	88.0	20.0	1.7	4.4	2.7
HT Proteolytic, 207	41.2	90.0	20.0	2.2	4.5	2.1

Table III gives the data obtained with six bacterial enzyme preparations all from the same organism but not necessarily produced under the same cultural conditions. As a group these preparations are much more potent than

those listed in the previous table. According to the assay in Northrup Units supplied by the manufacturer the first two preparations listed are about double the strength of the other four, about 930 to about 420 NU/g. respectively. None of our assays agrees very well with these values. The ratios of the values obtained by our assays are in fair agreement with those for the other bacterial enzymes, but even here there are over twofold differences.

Data for the new fungal enzymes tested are given in Table IV. Keratinase which is produced by *Streptomyces fradiae* is included for convenience. Considerable activity is shown by these preparations, but they are not as potent

TABLE IV

COMPARISON OF THE ACTION OF SOME FUNGAL ENZYMES ON GELATIN AT pH 7.0

Enzyme	Code	Assays			Ratios		
		Formol Titration meq-N/g	Viscosity Units/g	Liquefaction $\frac{1}{\mu g/l}$	V/FT	V/L	FT/L
Fungal Protease	PB	79.9	29.0	33.3	0.4	0.9	2.4
Rhozyme PH	RH	34.3	14.5	16.6	0.4	0.9	2.1
Prolase 40	WC	31.8	12.5	10.0	0.4	1.3	3.2
Keratinase	IM	70.7	32.0	50.0	0.5	0.6	1.4
Keratinase (at pH 8.5)	IM	125.1	51.0	50.0	0.4	1.0	2.5

as several of the bacterial enzymes. The ratios of the various activities are fairly consistent, but some differences are apparent. The optimum pH for the keratolytic activity of Keratinase is 8.5 to 9.0, and it is also more active on gelatin at pH 8.5 than 7.0 except when measured by the liquefaction technique.

Table V gives the data obtained using enzymes of plant and animal origin. Bromelin has comparatively high activity, but papain is quite weak. Special Diastase 160 is a mixture of plant and animal enzymes, mostly amylolytic, and has only weak action on gelatin. Crystalline trypsin digests gelatin more actively than any of the other enzyme preparations tested. However, the increase of activity over the noncrystalline pancreatic preparations varied markedly depending upon the assay method. Again there was much discrepancy between the ratios of the values for the three assays. It is well known that the optimum pH for the action of pancreatic enzymes is above 8.0. Therefore, in addition to those reported in this Table, assays were run on the three tryptic preparations at pH 8.5. The increased activities at pH 8.5 over their activities at pH 7.0 varied from 8 to 30%.

TABLE V

COMPARISON OF THE ACTION OF PLANT AND ANIMAL ENZYMES ON GELATIN AT pH 7.0

Enzyme	Code	Assays			Ratios		
		Formol Titration meq-N/g	Viscosity Units/g	Liquefaction $\frac{1}{\mu g/l}$	V/FT	V/L	FT/L
Bromelin	MC	77.7	67.0	100.0	0.9	0.7	0.8
Papain	NB	14.2	7.7	6.3	0.5	1.2	2.3
Special Diastase 160	MC	6.7	0.7	1.3	0.1	0.6	5.4
Viokase	VC	46.0	3.5	16.0	0.1	0.2	2.9
Trypsin (4 × USP Pancreatin)	NB	50.8	2.5	25.0	0.1	0.1	2.0
Trypsin (crystalline)	NB	435.2	750.0	500.0	1.7	1.5	0.9

TABLE VI

THE EFFECT OF pH AND ACTIVATOR ON THE ACTION OF BROMELIN AND PAPAIN ON GELATIN

pH	Sulfite* %	Bromelin (MC)		Papain (NB)	
		Formol Titration meq-N/g	Liquefaction $\frac{1}{\mu g/l}$	Formol Titration meq-N/g	Liquefaction $\frac{1}{\mu g/l}$
5.0	None	178.8	66.6	22.4	0.6
5.0	0.4	46.1	33.3	4.0	0.1
7.0	None	77.7	100.0	14.2	6.3
7.0	0.4	81.6	83.3	4.6	20.8
8.5	None	—	28.6	—	5.0
8.5	0.4	—	83.3	—	16.6

*As sodium metabisulfite on total solution basis

The effects of controlling the pH at 5.0, 7.0, and 8.5 and the presence of sodium metabisulfite on the activity of bromelin and papain are shown in Table VI. According to the formol titration assay bromelin was 2.3 times more active at pH 5.0 than at pH 7.0. Sulfite exhibited a marked inhibitory action at pH 5.0 and a slight activation at pH 7.0. We were unable to obtain reliable values at pH 8.5. Results using the liquefaction assay show that the highest activity for bromelin without activator was at pH 7.0. The addition

of sulfite resulted in a 50% decrease of activity at pH 5.0, a slight decrease at pH 7.0, and a threefold increase at 8.5.

Papain was much less active than bromelin. According to the formol titration assay the highest activity of papain was at pH 5.0. Sulfite had a decided inhibitory effect at both pH 5.0 and 7.0. Results with the liquefaction assay showed that there was very little activity at pH 5.0 and only moderate activity at pH 7.0 and pH 8.5. With this assay the presence of sulfite caused an inhibition at pH 5.0 but marked activation at pH 7.0 and pH 8.5.

The comparative depilatory activities of these enzymes have been determined and reported previously (1, 2, 3). The unhairing scores of the enzymes are compared in Table VII with their relative ranks according to the three

TABLE VII

COMPARISON OF THE UNHAIRING SCORES* OF ENZYMES WITH THEIR RELATIVE RANKS ACCORDING TO ASSAYS BY FORMOL TITRATION, VISCOSITY, AND LIQUEFACTION

Enzyme	Code	Unhairing Score	Formol Titration	Viscosity	Liquefaction
Trypsin (Crystalline)	NB	—	1	1	1
HT Proteolytic, 202	MC	1	3	2	3
HT Proteolytic "P"	MC	1	4	3	5
Keratinase (pH 8.5)	IM	1†	2	11	4
HT Concentrate, 4903	MC	2	7	5	4
HT Proteolytic, 1	MC	2	8	4	5
HT Proteolytic, 207	MC	2	13	6	7
HT Proteolytic, 210	MC	3	9	7	7
Protease L56-D	PB	3	14	9	6
Keratinase (pH 7.0)	IM	4†	7	12	4
Protease 15 Concentrate	RH	5	12	8	7
Trypsin (4 × USP Pancreatin)	NB	6	10	20	6
Protease 4511-3	WC	7	17	14	10
Fungal Protease	PB	8	5	13	5
Bromelin	MC	8	6	10	2
Rhozyme P11	RH	9	15	15	8
Prolase 40	WC	10	16	16	11
Papain	NB	11	19	17	13
Viokase	VC	11	11	19	9
Special Diastase 160	MC	12	21	21	15
Rhozyme H39	RH	13	18	18	12

*From Cordon *et al.* (2)

†Estimated from many tests. This enzyme was used on the basis of a keratinase assay (5) and cannot be compared on a weight basis with the other enzymes.

gelatin assays. Crystalline trypsin far outranks all other preparations in the gelatinase assays. However, because of its high cost, its use as an unhairing agent is precluded, and even the testing of its hair-loosening ability was considered impractical. When the methods of assay were tested in comparison with the unhairing activity, they were significant statistically. However, the magnitude of the relationship was not great enough to permit dependable predictions. In the best case tested (bacterial enzymes against viscosity) it was only possible to estimate the unhairing activity within ± 40% with 95% confidence.

DISCUSSION

Although enzymatic attack on gelatin undoubtedly consists of cleavage of the molecule, this is apparently accomplished in different ways by different enzymes. If breakdown always consisted of the rupture of the same linkages, the values obtained by titrating the acid produced and the change in viscosity or the ability to form a gel should be constant. Obviously this is not so. Perhaps changes somewhat analogous to those taking place when collagen is converted into gelatin by heating in water are produced by some of the enzyme systems.

At first thought it might appear that the viscosity assay and the liquefaction assay are measures of the same property of the gelatin. Liquefaction, however, as we define it, is really the ability of the protein to form a gel, and this is dependent on its ability to form a lattice network, a property quite different from viscosity. The data presented are replete with examples of enzyme preparations exhibiting the three different activities at different levels. Furthermore, alteration of the reaction conditions may or may not affect the three types of activity to the same extent when the pH is raised. A case in point is the change observed with Keratinase when the pH is raised from 7.0 to 8.5. This causes a 1.8-fold increase in activity as measured by formol titration, a 1.6-fold increase in activity as measured by viscosity, but no change in activity as measured by liquefaction.

Further evidence that different activities of the enzyme preparations are measured by the different assays is shown by the effects of changing the pH and adding a reducing agent to bromelin. pH 5.0 appears to be at or near the optimum for the action as measured by formol titration, whereas 7.0 is near the optimum as measured by liquefaction. Why sulfite should cause such a pronounced inhibition at pH 5.0 is not readily apparent unless the scission of the disulfide bond in bromelin leads to its complete conversion to the S-sulfo compound (4). Here again the effect is twice as pronounced with the formol titration as with the liquefaction assay.

The lack of correlation between hair loosening and any of the gelatinase assays clearly rules out this type of procedure for general use in studies of

the enzyme unhairing of hides and skins. However, if a single enzyme preparation were under investigation, one of the methods might prove useful for following its activity in unhairing studies.

SUMMARY

Twenty-one enzyme preparations were tested by three different assay procedures for their ability to attack gelatin. Results with the different procedures were quite variable. It was concluded that the different assay procedures measure different activities of the enzymes. No correlation was found between hair loosening and any of the assays.

ACKNOWLEDGMENT

The authors wish to thank Mr. C. E. Land of the Miles Chemical Company for supplying the viscosity assay method used and several of the enzyme suppliers for donating their products.

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FATLIQUORING

DISCUSSION OF A PAPER BY C. L. ORNES

On June 20, 1960, at the Fifty-sixth Annual Meeting of ALCA, held at the Lake Placid Club, N. Y., Conrad L. Ornes presented a paper entitled "The Influence of Type of Oil, Degree of Sulfation, and Neutralization Level of Fatty Acids on Some Physical Properties of Shoe Upper Leather." This paper was printed in the July, 1960, number of JALCA. The discussion that followed the presentation is now printed here.

DISCUSSION

BERNARD A. ROLL (Atlas Refinery): Thank you, Mr. Ornes, for this excellent work which, to the best of my knowledge, is the first attempt to base conclusions on sound statistical techniques, rather than mere subjective observations, in the study of leather characteristics as a function of fatliquor penetration.

Your conclusion that sulfated sperm is a more efficient softener than sulfated castor or sulfated cod probably agrees pretty well with practical tannery experience. Since the sulfated castor you were working with contained 77% free fatty acids, while the sulfated sperm contained only 15% free fatty acids, would it therefore not follow that you were really comparing ricinoleic acid and the monoglyceride of ricinoleic (in the case of the sulfated castor) against the di- and triglycerides of the sulfated sperm oil? That is, should it not be emphasized that your conclusion regarding the softening power of castor relative to sperm and cod might only be valid in the case where these respective oils were hydrolyzed to the extent indicated by the analyses of the sulfated oils with which you were concerned?

MR. ORNES: That point is well taken, and in committee meetings of the representatives of the industry-sponsored project that point was also brought up. It was unfortunate perhaps that the castor oil had such an unusually high percentage of free fatty acids.

ROSS G. DONOVAN (The Collis Leather Company): Is there any independent evidence that the three-factor interaction is a satisfactory estimate of error?

MR. ORNES: Your question is whether the three-factor interaction measures the error sufficiently well?

MR. DONOVAN: Or that it may be significantly different.

MR. ORNES: Well, there should be two duplicate specimens, in measuring true error. I think you will find in a large design such as this, in which we have grouped all the remaining interactions, which includes the error term as well, if error has occureed at all, we have erred on the safe side. We have divided the significant variance produced, by a larger term than we would have had to divide by if we had separated the two duplicate specimens. It must have been included within that variance in the three-factor analysis.

MR. DONOVAN: But if the three-factor analysis interaction itself were significant, that would invalidate the two-factor interaction tests, would it not?

MR. ORNES: None of the other interaction variables, you notice, were significant. If the two-factor variables are insignificant, I think you will rarely find that the three-factor variables are.

ARTHUR HIRSCH (Albert Trostel & Sons Co.): A very interesting point is that various fatliquor compositions appear to have a significant effect on the ability of leathers to corrode metals. (I am speaking primarily of mechanical leathers.) I wonder if this particular angle should not be included in a study of this kind.

MR. ORNES: Your point is that with the increased fatty acid level, of castor particularly, the corrosive effect produced would be important?

MR. HIRSCH: Yes.

MR. ORNES: That is probably true. However, since it was pointed out that the fatty acid level of the castor oil was abnormal to begin with, I don't think we would need to worry about that too much. In fact, in the follow-up experiments in which we put the same oils on an equal fatty acid basis, for example, we found relatively little difference among the three oils.

DR. ROBERT STUBBINGS (Lehigh University): Was all this work done on an acid-tanned chrome leather, or were studies made of other types of side leather?

MR. ORNES: Well, of course, this was one experiment involving 36 variables on a uniform lot of leather.

DR. STUBBINGS: What was the leather—the basic characteristic of the leather?

MR. ORNES: It was a normal side upper leather, chrome followed by chrome retan.

DR. STUBBINGS: Was it an acid chrome leather or a masked chrome leather?

MR. ORNES: This was an acid chrome leather, I believe. The significance of masked versus the ordinary acid did come up in later studies, but it was not a part of this one.

DR. STUBBINGS: You have made other studies, then, of other types of side upper leather, but this study that was shown this morning is only fatliquoring directly on blue chrome stock, without dyeing?

MR. ORNES: Yes. Later experiments also included the other effects.

MR. ROLL: Will these be published?

MR. ORNES: As soon as they are released to the industry, yes.

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EMPLOYMENT NOTICES

WANTED: Chemist for a large midwest upper leather tannery. Must be able to set up pilot-plant testing program. Opportunity excellent. Replies will be kept confidential. Write to Box R-152, c/o Secretary, American Leather Chemists Association, University of Cincinnati, Cincinnati 21, Ohio.

ABSTRACTS

Hydrothermal Denaturation of Collagen. K. H. Gustavson. *Nature*, **188**, 419–20 (1960).—A reply to an article by B. Rigby and J. Spikes (*Nature*, **187**, 150 [1960]) recalling the author's prior claim to the discovery of the occurrence of gradual shrinkage and labilization of mammalian collagen in water at 40° to 45°C. C.L.D.

Radioactivity in Wool. R. Kulwich, L. Feinstein, R. Decker, C. Golumbic, M. Hourihan, and C. Terrill. *Nature*, **188**, 511 (1960).—The γ-ray activity of clean wool is only a small fraction of that found in grease wool, which may contain 5% to 40% dirt content. The bulk of the content of grease wool emitting γ-rays is therefore associated with its impurities. It is suggested that γ-ray measurements, which can be made non-destructively in a few minutes, may serve as an index predicting the percentage of impurities present in grease wool and the relative proportions of the major impurities. C.L.D.

Proton Magnetic Resonance of the Water Phase of Gelatin Gels. E. Odeblad. *Nature*, **188**, 579 (1960). C.L.D.

Protein Catabolism. H. Walter. *Nature*, **188**, 643–45 (1960).—A review of the mechanism of protein catabolism is presented. It is pointed out that protein degradation is not simple proteolysis. Specific energy requirements for protein catabolism are indicated. Intermediates are shown to be involved in protein degradation, and it has been suggested that these might be similar to some of the intermediates known to participate in protein biosynthesis. C.L.D.

Hydroxyproline in Primary Cell Walls of Higher Plants. D. Lamport and D. Northcote. *Nature*, **188**, 665–66 (1960).—Proteins containing hydroxyproline have been found in the cell walls of sycamore and bean, but not in the cell contents. The percentage of hydroxyproline in the wall protein of sycamore cells (12.9 g. anhydro residues per 100 g. protein) is similar to that of mammalian collagens, but the amino acid analysis shows no other similarities. C.L.D.

The Presence of Magnesium and Aluminum Ions in Technical Sumac Extracts. E. Heidemann and H. Kröll. *Das Leder*, **11**, 201–5 (1960).—During adjustment of pH of technical sumac extracts a yellow, cheesy precipitate always formed near the neutral point. This precipitate was caused by the presence of magnesium and aluminum ions. Precipitation started at pH 6.5 and was complete at about pH 8; at higher pH values there was some resolution. This type of precipitate was given only by sumac extracts, either Sicilian or staghorn, and was not caused by mineral matter on the leaves. Ex-

tracts from carefully washed leaves gave the same results. The magnesium contents of the ashes of extracts prepared by leaching leaves (3 times) with water at 50°C. were 4.87%, 4.61%, and 11.68% resp. for staghorn, Sicilian, and a technical sumac extract. Corresponding values for aluminum were 0.85%, 0.61%, and 0.62%. The precipitate was found only in aqueous extracts, not in extracts made with organic solvents, and only in concentrated solutions. It was peptized by water and therefore was very difficult to purify by washing or reprecipitation. It would redissolve completely in acid only if acid was added soon after precipitation. On standing, even during the time required for centrifugation, there was an increase in the fraction insoluble in acid. As standing time increased, the amount of acid required for resolution increased. Dilution of sumac extracts also caused precipitation. For example, if a 20°–25°Bé clear extract was diluted 20-fold, a fine precipitate formed that could be separated by centrifuging at 400 rpm for 2 hr. The precipitate redissolved if the dilute solution was concentrated. If the precipitate was removed and the clear solution was concentrated, another precipitate did not appear on dilution. The dry "dilution" precipitate contained only 4% of metal cation, whereas the precipitate formed by neutralization contained 53%. The aluminum content of the former, dilution precipitate, was much greater than that of the latter, but the magnesium content was slightly lower. When sumac extract was stirred into 96% alcohol, a precipitate formed leaving the tannin in solution in fairly pure form. The precipitate contained the magnesium and aluminum salts and was nonhomogeneous, as was shown by the blurred paper chromatographic spot. These nontannins were not removed by cold extraction of sumac leaves. Acid hydrolysis of the total extract showed the presence of much more sugar than was found in purified extracts. For Sicilian sumac, tannin content is maximum at the time of maximum leaf development, but for staghorn sumac it is maximum when the leaves begin to turn color. The magnesium content of leaves increased beyond the time of maximum tannin content. Most of the magnesium was in the leaf residue; the amount in the chloroform extract (chlorophyll) was small. Other plant parts did not contain magnesium. Magnesium activates the enzyme that acts on phosphorylated substrates. I.D.C.

Trends for Economy in the Leather Industry and Means for Its Accomplishment. G. Classen. *Das Leder*, **11**, 189–95 (1960).—A discussion of the various methods now in use to reduce tanning time and to increase profits. I.D.C.

Tanning with Vegetable and Synthetic Tannins. V. The Effect of Different Acid-Salt Systems on Tannage with Different Types of Vegetable Tanning Materials. H. Herfeld and K. Schmidt. *Das Leder*, **11**, 195–200 (1960).—The type of experimental tannage used in an investigation of the effect of acid-salt mixtures on wattle tannage (abstract, *JALCA*, **55**, 647 [1960]) was repeated to include tannages with wattle, quebracho (lightly sulfited in the laboratory), chestnut, oakwood, myrobalans, and sumac extracts. The salts were sodium chloride, ammonium sulfate, and the sodium salts of formic, lactic, and acetic acids, and were used at only one concentration, 0.2 equivalents per liter. One series of liquors was adjusted to pH 4.5 and another to 3.5 with hydrochloric or acetic acid or with sodium hydroxide. Liquors containing salts of organic acids were adjusted usually with hydrochloric acid. As in the previous work fresh solutions were made for each increase in tannin

concentration. The total number of tannages was 72. The results showed that the conclusions regarding influence of acid-salt systems on tanning progress and leather properties, found earlier for wattle tannage, also generally held for the other tanning materials. The presence of larger amounts of salt during tanning improved constancy of pH, repressed swelling, resulted in more flexible leather with greater stretch, and favored penetration, but it decreased tannin fixation and made the behavior of the leather to water worse. The effect on leather color was not uniform. Stather, Reich, and Uberla (abstract, *JALCA*, **55,** 657 [1960]) found somewhat similar effects when they used large amounts (60%–100%) of exchange syntans in tanning. There is a question whether the unfavorable influences were caused by organic syntans or by the appreciable amounts of salt that were present. Modified vegetable tannins, syntans, and lignin extracts advisably should have low salt contents. **VI. The Effect of Different Acid-Salt Systems on Hot Pit Tanning (Tempering).** *Ibid.*, 222–26.—The effect of a high salt content of the hot pit liquors after a normal tannage in liquors with a low salt content was investigated by treating 850-g. portions of leather in 3-l. portions of hot wattle extract liquor containing 16.4% of pure tannin and 0.3 equivalents per liter of various salts. The leathers were prepared by tanning cattlehide in a pilot rocker–two suspender system until penetration was complete and the degree of tannage was 61. The liquors were at 40°C. and had a pH of 3.5. The salts were sodium chloride, ammonium sulfate, and sodium formate and acetate; pH adjustments (to 3.5) were made with hydrochloric, formic, lactic, and acetic acids. After 5 days in the hot pit vats, the leathers were thoroughly washed for 48 hr. in a number of changes of water, dried, conditioned, and tested. The pH values changed only slightly; they ranged from 3.48 to 3.60 before, and from 3.50 to 3.64 after, the tempering operation. The salt-acid additions had no effect on color of the leather or its stretch, softness, or flexibility. Degree of tannage was not altered except with acetic acid. About 5 times as much acetic as formic or lactic acid was required for pH adjustment in the presence of inorganic salts. This large amount of undissociated acetic acid had a hydrotropic effect on the hide; it set free tanning-active groups that tended to form coordination bonds with vegetable tannin and therefore to raise the degree of tannage. These leathers had low strengths. Acetic acid is not suitable for acidifying tempering liquors. This confirms reports of other workers; however a reported bad effect of lactic acid was not found here. A similar type of experiment was conducted with wattle, quebracho, oakwood, and chestnut extracts acidified to pH 3.5 with formic and lactic acids and containing 0.3 equivalents per liter of ammonium sulfate or sodium formate. The chestnut extract was acid, so that pH adjustments were made with sodium hydroxide instead of with acid. No effects of the salts were noted except that the quebracho leathers were less reddish if salt was present in the tempering liquors, and the chestnut leather was brighter with sodium formate. Acetate, either as salt or as undissociated acid, must be avoided entirely in the hot pit liquors as it damages fiber structure. Other salts had no effect; however in practice they should be avoided because they would be carried back into the earlier tanning liquors where they would have an unfavorable effect on tanning speed, yields, and water absorption of the leather. I.D.C.

International Union of Leather Chemists Societies. Physical Testing Commission Report on Meetings Held at Copenhagen on July 29 and 30, 1960, and Publication of Proposed Methods. R. G. Mitton,

Chairman. *J. Soc. Leather Trades Chemists*, **44**, 491–505 (1960).—The Commission has approved the following methods in the form agreed by the Commission's members. At the conference in the U. S. A. next year it will be proposed that they be declared official.
I.U.P./11 Dynamic Waterproofness Test for Boot and Shoe Sole Leather
I.U.P./14 Measurement of the Waterproofness of Glowing Leathers
I.U.P./15 The Measurement of Water Vapor Permeability S.D.

Potentiometric Titration Studies on Solutions of Zirconium Oxychloride and Zirconium Sulfate in the Presence of Some Organic Reagents. D. A. Williams-Wynn. *J. Soc. Leather Trades Chemists*, **44**, 405–18 (1960).—This study was made for the purpose of obtaining information about the formation of zirconium complexes with various organic reagents including acetic and glycollic acid, glycine, and α, β, and γ-amino-n-butyric acid. Solutions of zirconium oxychloride and zirconium sulfate alone and with various molar ratios of the organic acids were titrated potentiometrically with alkali. The effect of the organic acids on the pH at which precipitation occurred was observed. An increase in this pH was taken as evidence for complex formation. From the results obtained it is concluded that with zirconium oxychloride coordination with carboxyl groups occurs over the pH range 1.0 to 3.7. There is no evidence of coordination with amino groups. Zirconium sulfate behaves quite differently with these acids; little or no coordination was found with either the carboxyl or the amino groups. Estimation of the amounts of organic acid coordinated shows no evidence of specific combining proportions, but the pattern is typical of a mass action relationship. There is no simple relationship between complex formation and the pK values of the acids investigated. S.D.

The Action of Perspiration on Leather. Part 1. The Action of Lactic Acid on Chrome Leather. J. H. Bowes and J. A. Moss. *J. Soc. Leather Trades Chemists*, **44**, 419–36 (1960).—The factors effecting the extraction of chrome from leathers by a solution of sodium lactate and sodium chloride have been investigated. The amounts of chrome extracted are dependent on the time and temperature of extraction, but eventually an equilibrium is reached which depends on the relative amounts of lactate and leather present. It is concluded that the chrome distributes itself between the leather and the solution according to the number of chemical groups present in each capable of complexing with the chrome. Shrinkage temperature of the leathers decreased evenly with removal of chrome, indicating that all the chrome fixed contributed equally to the hydrothermal stability. A procedure is suggested for tests of the relative stability of the chrome-collagen complex in different leathers. The effects of pH and different anions on the extraction are discussed. S.D.

Leather Research in Holland. P. J. van Vlimmeren. *J. Soc. Leather Trades Chemists*, **44**, 463–67 (1960).—A survey.

Statistical Techniques in Production—A Case History. G. A. R. Taylor. *J. Soc. Leather Trades Chemists*, **44**, 467–77 (1960).—A detailed illustration is given of the application of simple statistical techniques to the problem of evaluation of leathers used in cycle saddles. S.D.

Gelatine, The Current Position. A. G. Ward. *J. Soc. Leather Trades Chemists*, **44**, 505–18 (1960).—A review, 30 references. S.D.

The Physical Properties of Leather Finish Films. W. O. Nutt. *J. Soc. Leather Trades Chemists*, **44**, 519–24 (1960).—A discussion of the relationship between finish performance and the constitution and physical properties of the finish films. S.D.

Leather as a Heat Insulating Material. A. Sen. *J. Indian Leather Technologists' Assoc.*, **8**, 289–302 (1960).—A review with 18 references. H.B.M.

Utilization of Myrobalans in Combination with Synthetic Tannins. N. Viswanathan. *J. Indian Leather Technologists' Assoc.*, **8**, 303–7 (1960).—Laboratory tests indicate that best over-all results, as to elimination of sludge and quality of leather, are obtained with a mixture containing 75% myrobalans, 15–20% of replacement syntan and 5–10% of auxiliary syntan. H.B.M.

Metals in the Leather Industry—Aluminum. G. W. Douglas. *Tanner* (India), **15**, 153–54 (1960).—Aluminum can be used to advantage where it will not be exposed to a highly acid or alkaline environment. H.B.M.

The Technique of "Chinese Chrome". A. K. Mukherjee. *Tanner* (India), **15**, 145–48 (1960).—The processes used by Chinese tanners in Calcutta are described. The extreme cheapness of such leather, combined with good quality, is attributed to low labor costs (largely family labor), low capital investment (very little machinery), and rapid turnover. The entire process takes only 8 days: 1 soak; 2 lime; 1 delime, pickle, Cr tan; 1 shave; 1 wash, dye, fatliquor, dry (in the sun on a tin roof!), dip, pile; 1 stake, tack out, apply first season (to damp leather on the tacking boards); 1 or 2 trim, apply second season, sun-dry, glaze, stake, iron. Liming is done in a running lime, with lime, Na_2S, and NH_3, discarding 1/3 of the liquor after each pack. Deliming (partial) is done with $(NH_4)_2SO_4$ or acetic acid; bating is omitted. Pickling and Cr tanning are conventional, using molasses-reduced Cr liquor. The leather is not neutralized, but it is washed thoroughly before dyeing. Dyeing and fatliquoring are done at room temperature, using fish oil (often low-grade) and a little sulfated oil. H.B.M.

Purification of Waste Waters from European Tanneries. H. Scholtz. *Leder Kurier*, **4**, 159–65, 188–96 (1960).—Proper purification of tannery wastes is becoming of more importance in European countries. Published methods for treating wastes are not good because they are based on analyses of poor samples, do not take into account all substances that may be present, or prescribe excessive treatment that causes unnecessary expense. These wastes are not of one uniform composition; 11 main types are listed based on type of leather and tannage. In only about 10% of 800 tanneries could the waste be mixed directly with city sewage. Waste that is discharged into city sewage must not form too much sludge in the sewers or corrode them and must cause no odor or poisoning. A system is outlined for treating 11,000 cubic meters per day of combined waste from two chrome upper leather tanneries and a sole

leather tannery. The waste was collected in a circular tank large enough to hold 50% of the daily volume. Efficient stirring in this mixing tank was important. Soak liquors which contain considerable salt, and other liquors, were dense and tended to pass through the tank without dilution, so that special paddle mixers were required. Sludge in the mixing tank was about 3% of the water volume and did not vary much from day to day. From the mixer the waste was pumped to a chemical section for treatment with ferrous sulfate, oxidation, and flotation removal of hair. The ferrous sulfate was dissolved at constant temperature to assure constant concentration, then pumped in the required amount into the stream of waste water going to a "palette" paddle for oxidation. Ferrous sulfate was used because it could be obtained very cheaply as a by-product of other industries. It removed most of the organic matter, but a small amount of colloidal ferrous sulfide remained in solution and colored it black. The color could be prevented by precise adjustment of pH, but in a plant pH adjustment would be too difficult because pH fluctuated daily between 6.8 and 7.5. There was no color if a trivalent iron salt was used, but this material was too expensive; oxidation in a paddle was cheaper. The oxidation required at least 20, and preferably 30, minutes. In the paddle there was little difficulty if wetting or emulsifying agents from dye or fatliquor wastes were present; but if mixing in the first tank was poor, protein matter was carried over to the paddle and interfered with the oxidation. From the paddle, after removal of the floating mat of hair, the waste entered settling tanks. Hair in the settling tank led to the formation of a dense, matted sludge that was difficult to remove. Sludge containing iron was not fermentable but was suitable for fertilizer. It was dewatered by filtration with suction after aging or warming (to 28°–30°) to avoid a slimy deposit that clogged the filter. Sludge not containing iron could be fermented to produce methane for fuel. The treating process must be modified to suit the particular conditions at the given tannery, and considerable saving is possible by proper adjustments.

I.D.C.

Preparation and Properties of Vulcanizable Waterproof Leather. K. Rosenbusch and G. Hudec. *Das Leder*, **11**, 237–46 (1960).—Waterproof leather is side upper leather that has been either chrome tanned or vegetable retanned, then fatliquored normally and stuffed with grease to contain 17–23% extractable fat. If leather contains more than 12% or 14% fat, it cannot easily be vulcanized to rubber, and finish coatings will not adhere well. At the high vulcanization temperature the fat becomes liquid and moves to the surface and prevents a firm union. Also leather with a high fat content has poor heat insulation and may cause frozen feet if worn in shoes under severe conditions. If the fat content is less than 10%, adhesion and insulation are good. The tanner must compromise between the softness and waterproofness obtained with a high fat content and heat insulation and ability to be readily finished or vulcanized which require a low fat content. Impregnating agents are better than fat. Such materials, however, must be able to penetrate the finish coat of leather without swelling or loosening it. To determine conditions for obtaining good waterproof leather, specimens were prepared with increasing amounts of train oil (9.8–56%) Derminol-Öl HlF (10.6–43.6%), and an impregnating agent (9.9–22.1%). The last consisted of 3 parts by weight of Primenit F and 1 part Imprägnierungsmittel LT6B. Extractable matter in these leathers was determined, after aging for 3 days, by extraction with petroleum ether. Static water absorption was determined by cutting a specimen, coating

the cut edges with collodion, and weighing before and after shaking it for 15 minutes in water at 20°C. Dynamic water absorption was determined with a Bally-Penetrometer. Adhesion of leather to rubber after vulcanization was tested on bonds made by coating pieces of leather and rubber (8 x 15 cm.) twice with special vulcanizing adhesive, drying, then heating to temperatures ranging from 140° to 250°C. in a press with the rubber to the hot plate. Bond strength was tested in a Schopper tester on 1 x 5 cm. specimens after separating the pieces at one end with benzene. At 230°C. the leather began to be damaged, so further vulcanizing was done at 200°C. It was necessary to roughen the leather surface in order to get a satisfactory bond. Heat transfer was measured by putting a disc of leather, split to uniform thickness, between 2 reservoirs, the left filled with mercury at 60°C. and the right with mercury cooled by running ice water. When the mercury in the left vessel cooled to 37°C., its temperature was recorded at regular time intervals for 30 minutes to obtain a cooling curve. The water absorption of impregnated leather was quite low, that of leather treated with Derminol was medium, and that of leather treated with train oil was high unless a large amount was present. Bond strength of impregnated leather to rubber remained high with increase of impregnating agent, but strength decreased with increasing amounts of fat. Transfer of heat through the leather increased as the amount of fat in the leather increased; the impregnating agent gave better insulation than fats. A new type of vulcanizable, low-fat, impregnated leather was made and compared with classical waterproof leather. The leathers were prepared as follows: Hides split to 2.5 mm. were retanned to contain 1.5% Cr_2O_3 and 18% of wattle-quebracho (1:1) tannin, dyed black, fatliquored with Derminols, and treated with fixing agents. The leather was divided into 3 parts, and one part (Lot A) was tacked out, dried, and finished with binder and glaze coats. Another part (Lot B) was finished as Lot A then given 2–3 brush coats on the grain of Primenit F in 4 parts of "Shell" petrol, and 2–3 brush coats on the flesh of a mixture of 3 parts Primenit F, 1 part Imprägniermittel LT6B, and 16 parts of petrol; 15–20% was taken up. Lot C, before finishing, was coated on the under side with a mixture of Derminols, train oil, tallow, and degras, then finished as for Lot A. The amounts of extractable fat were 4.65%, 5.59%, and 16.30% resp. for Lots A, B, and C. Penetration times in the Bally-Penetrometer were 9, 363, and 24 minutes resp. for A, B, and C. Heat transfer was lowest through leather with the least extractable fat. Good adhesion to rubber was obtained only when extractable fat was less than 12%. Lot C would not adhere to rubber. If leather was high in fat, when the pieces were pulled apart the adhesive was all on the leather and the rubber was smooth. If the leather had been impregnated or was low in fat, the torn surfaces were not clean, and adhesion was great enough to tear the material. I.D.C.

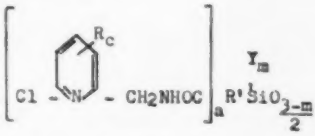
PATENTS

Waterproofing. Brit. Pat. 834,962. Union Chimique Belge S.A. Appl. April 1, 1958.—Porous materials, e.g. textiles, leather, paper, cardboard, bricks, tiles, asbestos and cellular cements, terra-cotta and earthenware articles and tarred felt, are rendered water-repellent by treatment with a composition comprising an aqueous emulsion of an organopolysiloxane, a pigment and a thermoplastic resin, e.g. a polymer or copolymer of vinyl chloride or acetate, acrylic

or methacrylic acid or an ester or nitrile thereof, styrene, butadiene, isoprene, chloroprene, isobutene, ethylene or propylene (*see* Group IV (a)). Pigments specified are oxides of Fe, Cr and Ti; chromates of Pb and Zn; sulphides of Zn and Cd; sulphates of Ba and Ca; and powdered Al, Zn and bronze. The emulsion may also contain siloxane solvents, thickeners and emulsifying agents. Very porous materials are preferably pre-treated with an acrylic or other synthetic resin or varnish so that the majority of the pores become blocked. The compositions when applied to the material to be treated air dry rapidly. Examples are given. Specification 806,226 is referred to.

Leather Substitutes. Brit. Pat. 834,938. Goppinger Kaliko-und Kunstleder-Werke G.m.b.H. Appl. May 27, 1957.—A leather substitute having a mat surface resembling that of chamois leather is produced by coating a foundation with a mixture of polyesters and polyisocyanates containing wood meal or ground cork, leather, cellulose or asbestos, and subjecting the coated foundation to elevated temperatures to form a high molecular polyurethane coating. The foundation which may be a woven or knitted fabric, web, felt or paper, foil of natural or synthetic substance such as natural or synthetic rubber, vinyl polymers or copolymers, polyamides, cellulose esters or ethers, or imitation leather, may be mechanically roughened, or provided with a mat impression, coated with a polymethane varnish and provided with an embossed grain, or may be coated with other high molecular natural or synthetic materials such as natural or synthetic rubber, vinyl polymers or copolymers, cellulose esters or ethers, polyamides or a carbamide resin, which may be roughened, prior to the application of the filled coating. The components of the polyurethane may be a low molecular polyester of a bivalent alcohol and a bivalent aliphatic dicarboxylic acid, and an aromatic polyisocyanate, e.g. toluylene diisocyanate, or a polyisocyanate partly condensed with a polyester, may be applied as a solution in a suitable non-aqueous and non-alcoholic solvent, e.g. butyl or ethyl acetate, which is evaporated prior to the formation of the polyurethane.

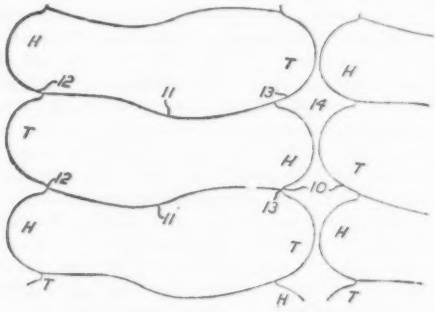
Waterproofing Textiles &c. Brit. Pat. 835,561. Midland Silicones Ltd. Appl. April 16, 1958.—Organic fabrics, such as cotton, viscose, acetate, nylon, polyacrylonitrile, polyester fabrics, paper, leather, linen, wool and silk, are rendered water-repellent by treatment with a copolymer of from 0.1 to 99.9 mol. per cent of units of the formula



in which R is an aliphatic hydrocarbon (h.c.) radical of 1–3 C atoms, *c* is 0, 1, 2 or 3, *a* is 1 or 2, R' is a di- or tri-valent saturated aliphatic or cycloaliphatic h.c. radical containing at least 2 C atoms, Y is a monovalent h.c. or fluorinated h.c. radical and *m* is 0, 1 or 2, and from 99.9 to 0.1 mol. per cent of conventional siloxane units, or with a composition comprising from 0.1 to 95% (by weight) of (1) a siloxane of the above formula or copolymer thereof (as above) and from 5 to 99.9% of (2) a siloxane having an average from 1 to 3 substituents selected from H atoms and monovalent h.c. and halogenated

h.c. radicals per Si atom (*see* Group IV (a)). Those copolymers containing at least 30 mol. per cent of amidomethylsiloxane units are water-soluble and can be applied from aqueous solution whereas aqueous emulsions or organic solvent solutions are used for other copolymers and compositions. The fabric may be treated by the mixture of siloxanes (1) and (2), or first by (1) or (2) and then by (2) or (1). In any case, the fabrics after application of the siloxanes are heated from 1 to 10 minutes to give durable finishes. Additional curing agents, other resins such as melamine- or urea-formaldehyde resins, and other additives for decreasing slip of the fibres or for imparting soil resistance, e.g. colloidal silica, may also be added or be applied separately to the fabrics. Examples are given.

Cutting-Machines. Brit. Pat. 836,388. Collinson's Patents Ltd. Appl. Dec. 22, 1958.



—In a machine of the kind described in Specifications 579,343 and 693,507 in which a sheet of leather, rubber or other material is placed on a multiple knife and relative movement takes place between the knife and a co-operating member, e.g. a roller to impale the sheet material on the knife so that a multiplicity of soles are cut out simultaneously, the knife comprises transverse knives 10 to alternately cut a toe and heel and longitudinal knives 11, each of which is adapted to cut the outside edge of one sole and the inside edge of its neighbour. The knives 11 abut against the knives 10 at 12 and 13. T and H indicate the toe and heel ends of a sole-shaped knife element of the multiple knife. The cut soles and the waste 14 may be ejected from the multiple knife by ejectors such as described in Specification 678,289 or 702,324, or by means of rubber pads.

Fatliquoring Leather. Brit. Pat. 836,808. Du Pont de Nemours & Co., E. I. Appl. July 5, 1956.—The complex compounds of the Werner type in which a trivalent nuclear chromium atom is co-ordinated with carboxylic acids groups having at least 10 carbon atoms as described in the parent Specification are applied to the leather before, or preferably after, conventional fatliquoring. Conventional fatliquoring oils named are neat's-foot, coconut, fish, sperm, castor, mustard seed, tea seed, lard, corn, olive, linseed, rapeseed, soya bean and tallow oil which may be emulsified and/or sulphated. The pH is adjusted to 3–3.5 by the addition of a monobasic acid such as formic acid before adding the chromium complex. In examples: (1) chrome-tanned cowhide is split, retanned with

Quebrancho extract, fatliquored with sulphated neat's-foot and cod oil and subsequently egg yolk, dyed, then treated with formic acid and finally stearato chromic chloride in aqueous iso-propanol; (2) cowhide is tanned and retanned as in (1), fatliquored with sulphated neat's-foot and fish oil and egg yolk, dyed and finally treated with stearato chromic chloride in aqueous iso-propanol; (3) steerhide is tanned in a manner related to (1), retanned with a mixture of Quebrancho and wattle, dyed, fatliquored with cationic sperm oil, sulphated fish oil and emulsified fish oil and finally myristato chromic chloride.

Impregnated Paper and Leather. Brit. Pat. 835,597. Suddeutsche Kalkstickstoff. Werke A.G. Appl. March 29, 1957.—An improving agent for paper or leather comprises (i) a pre-condensation product of formaldehyde and either dicyandiamide or melamine, the pre-condensation product having at least one methylol group for each $—NH_2$ group, and (ii) an organic nitrogen compound condensable with formaldehyde, in the form of a salt with an inorganic acid or an organic carboxylic acid (the salt having an acidic reaction in aqueous solution) and in quantities of 15–35% (preferably 20%) by weight calculated on the weight of the mixture. In examples there are disclosed solutions in boiled water of the following mixtures, all of which are stated to be suitable for the impregnation of paper or leather:—(1) hexamethylol melamine, ureahydrochloride and starch; (2) pentamethylol melamine, dimethylol dicyandiamide and melamine monophosphate; (3) tetramethylol melamine and dicyandiamide mononitrate; (4) hexamethylol melamine and melamine monoformate, -chloracetate or -hydrochloride.

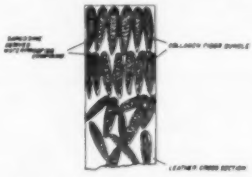
Process for the Production and Use of Novel Tanning Agents and Products Produced by Such Process. U. S. Pat. 2,944,046. L. Sellet, Saddle River, N. J., assignor to Jacques Wolf & Co., a corporation of New Jersey. Filed Sept. 23, 1952.—1. A process for the preparation of a modified amino-formaldehyde resin having cationic active properties and which is stable in water and will not heat polymerize or condense therein, comprising reacting at a temperature in the range of about 75°C. to about 110°C. (*a*) a water-soluble, amino-formaldehyde resin containing free unreacted methylol groups which impart instability to said amino-formaldehyde resin in water, said amino-formaldehyde resin being selected from the group consisting of urea-formaldehyde resins, thiourea-formaldehyde resins, guanidine-formaldehyde resins, acetylene diurea-formaldehyde resins, melamine-formaldehyde resins and dicyandiamide-formaldehyde resins with (*b*) a water-soluble amino base selected from the group consisting of urea, thiourea, guanidine, acetylene diurea, melamine and dicyandiamide, the water-soluble amino base being in an amount sufficient to react with all of the methylol groups of the amino-formaldehyde resin, said reaction being carried out at a pH of about at least 7.

Aqueous Latex Containing Copolymers of Chloroprene of 2,3-Dichloro-1,3-Butadiene and Vinyl Toluene. U. S. Pat. 2,852,481. P. R. Johnson, Wilmington, Del., assignor to E. I. du Pont de Nemours and Company, Wilmington, Del., a corporation of Delaware. Appl. March 7, 1955.—1. An aqueous alkaline latex of a copolymer adapted for use as a base for finishes for leather prepared by emulsion copolymerization of from 30 to 50 parts of chloroprene, 5 to 15 parts of 2,3-dichloro-1,3-butadiene and from 40 to 60 parts of vinyl toluene, in the presence of from 0.25 to 1 part of a long chain alkyl mercaptan.

Process for Dehairing of Skins with Ultrasonic Energy. U. S. Pat. 2,965,435. P. Renaut, Meaux, France, assignor to Realisations Ultrasoniques, a corporation of France. Filed June 25, 1958.—4. A method for unhairing skins comprising the following steps: immersing a skin into water comprising a wetting agent and an antiseptic substance; allowing said skin to swell in said water; removing said skin from said water; allowing said skin to lose a portion of the water it has retained; dipping said skin into water; and submitting said skin to ultrasonic energy of a frequency comprised between 10 kilocycles per second and 100 kilocycles per second.

Leather Impregnite. U. S. Pat. 2,967,165. M. Bailey, 36 Kenilworth Place, Brooklyn, N. Y. Filed Oct. 14, 1958.—1. A composition of matter for treatment of leather to render it resistant to penetration by mustard gas, lewisite and like vesicants which comprises about 21 to 26 parts by weight of a polyisobutylene having a molecular weight of about 80,000, about 21 to 44 parts by weight of paraffin wax, about 36.8 to 15 parts by weight of paraffin oil, and about 10.6 to 15 parts by weight of neat's-foot oil.

Waterproofing of Leathers. U. S. Pat. 2,964,425. R. J. Holzinger, North Merrick, and G. M. Whelan, New York, N. Y., assignors to Socony Mobil Oil Company, Inc., a corporation of New York. Filed Apr. 29, 1958.



—1. The method of making leather resistant to water penetration which comprises introducing into the leather a compound of the formula

$$R—N(CH_3)—CH_2—COOH$$

wherein R is an aliphatic acyl radical of 16 to 22 carbon atoms.

Process for Treating Wool and Other Fibrous Materials to Impart Water Repellency and Resistance to Shrinkage. U. S. Pat. 2,961,338. E. Robbart, 42 Bailey Road, Watertown 72, Mass. Filed May 7, 1958.—1. The method of treating wool in process having a regain in excess of about 12 percent on its dry weight, to render said wool water repellent and shrink resistant, comprising drying said wool until its regain is in an amount less than 10 percent on its dry weight but at least sufficient to react with an organo silicon halide to render said wool water repellent, and thereafter contacting said wool with vapors of an organo silicon halide until the wool is rendered water repellent.

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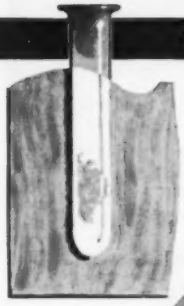

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